

## PCR Screening for Recombinase Activating Gene-1 null (RAG-1<sup>-/-</sup>) mice

### Principle:

The RAG-1 (and RAG-2) genes are located on the mouse chromosome 2. RAG-1 gene contains two exons. The mouse RAG-1 gene product contains 1040 amino acids and is coded for entirely by the second exon. The neomycin-resistance expression cassette disrupts the second exon between nucleotides 482 and 1837. The neomycin-resistance cassette is inserted in the opposite orientation to that of the RAG-1 gene. The wild type primer chosen for PCR screen starts at position 309 and stops at position 734. The wild type reaction fragment is 425 bp long. The neo cassette primer starts at position 1 and stops at position 464 and the reaction fragment is 1.1 kbp long.

### Primers:

1. RAG-1 wild type:  
sense: 5' ACTCAATTCTGACTCAACG 3'  
Antisense: 5' AACAGATGTCACAGGACG 3'
2. Neo: 5' AGAAAGTATCCATCATGGC 3'

### PCR:

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|-----------------|--|
| <u>Reaction</u> | 1. Genomic DNA (2: 1)  |
|                 | 2. Promega PCR Master Mix (2X; 10: 1)                            |
|                 | 3. Primers (100pmol/: 1; 0.5: 1 each)                            |
|                 | 4. PCR water (6.2: 1; to a final total reaction volume of 20: 1) |

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|----------------|---|
| <u>Program</u> | 1. 35 cycles- 94°C for 1 min., 49.5°C for 1 min., 72°C for 2 min. |
|                | 2. 1 cycle - 72°C for 7 min.                                      |
|                | 3. Hold at 4°C for 4.   |

### Expected bands on TBE agarose gel electrophoresis:

Wild-type band = 425 bp long.  
Knock-out band = 1.1 kbp long.

### Screening for double KO mice

This strategy may be used to detect the RAG-1 alleles in the double knock-out mice, as the neo primer in the RAG-1 screen and apoE screen are of different size.

### Reference:

RAG-1 deficient mice have no mature T and B lymphocytes; Cell, Vol. 68, 869-877, March, 1992

